Heavy Metals Uptake in Plant Parts of Sweetpotato Grown in Soil Fertilized with Municipal Sewage Sludge

George F. Antonious, Sam O. Dennis, Jason M. Unrine, and John C. Snyder

Abstract— Municipal sewage sludge (MSS) used for land farming typically contains heavy metals that might impact crop quality and human health. A completely randomized experimental design with three treatments (six replicates each) was used to monitor the impact of mixing native soil with MSS or yard waste (YW) mixed with MSS (YW +MSS) on: i) sweet potato yield and quality and ii) concentration of seven heavy metals (Cd, Cr, Mo, Cu, Zn, Pb, and Ni) in sweet potato plant parts (edible roots, leaves, stem, and feeder roots). Soil samples were collected and analyzed for total and extractable metals using two extraction procedures, concentrated nitric acid (to extract total metals from soil) as well as $CaCl_2$ solution (to extract soluble metals in soil that are available to plants), respectively. Elemental analyses were performed using inductively coupled plasma mass spectrometry (ICP-MS). Overall, plant available metals were greater in soils amended with MSS compared to control plots. Concentration of Pb was greater in YW than MSS amendments. Total concentrations of Pb, Ni, and Cr were greater in plants grown in MSS+YW treatments compared to control plants. MSS+YW treatments increased sweet potato yield compared to plants grown in native soil. Concentration of heavy metals in MSS amended soil and in sweet potato roots were below their respective permissible limits. However, monitoring heavy metals in soil and edible plants should be regarded as a requirement for the safe use of soil amendments in agricultural fields.

Keywords**—**biosolids, cadmium, lead, nickel, nutritional composition, sweet potato quality, soil conditioners.

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I. Introduction

Sweet potatoes earned U.S. producers about $395 million in 2008 [1]. It is the world's seventh most important food crop after wheat, rice, maize, potato, barley, and cassava [2]. Sweet potato roots are packed with β-carotene, sugars, vitamin c, phenols, and fiber. It is a major food crop in developing countries with a total world production of approximately 130 million tons [2]. Sweet potato ranks as the world's third most important starchy root crop, after cassava (*Manihot esculenta* Crantz) and potato, *Solanum tubersosum* L. [3].

Farmers, especially limited resource farmers, are continually searching for alternatives to synthetic fertilizers to alleviate the escalating production costs associated with the increasing costs of energy and fertilizers and the problems of soil deterioration and erosion associated with intensive farming systems. Agriculture is a major industry in the Commonwealth of Kentucky, where about 80% of the farmers are limited resource farmers. Recycling wastes would reduce dependence on synthetic fertilizers and provides amendments useful for improving soil structure and nutrient status [4]. The benefits of organic amendments to growth and yield of vegetables have been clearly demonstrated [5-7]. The increased production of municipal sewage sludge (MSS) in the U.S. has led many municipalities to consider its application to agricultural land as a means of sludge nutrient recycling. The U.S. Environmental Protection Agency (USEPA) promotes the use of municipal solids for land farming because it decreases dependence on chemical fertilizers and provides significant economic advantages. MSS, sometimes referred to as biosolids, contains organic matter, and macro- and micronutrients important for plant growth. Sixteen elements out of the ninety found in plants, known to be essential for plant growth, are present in biosolids [8]. In addition, the simultaneous use of soil conditioners to enhance soil physical, chemical, and microbial conditions could also enhance soil bioremediation [9, 10]. Soil incorporation of composted MSS usually results in a positive effect on the growth and yield of a wide variety of crops and promotes the restoration of ecologic and economic functions of soils.

Agricultural uses of MSS have shown promise for a variety of field crops (e.g., maize, sorghum, forage grasses) and production of vegetables (e.g., lettuce, cabbage, beans, potatoes, cucumbers) [11]. The organic matter content of composted MSS is high and its addition to agricultural soils often improves soil physical and chemical properties and enhances biological activities [7]. Most agricultural benefits of MSS compost application to soil are derived from improved physical properties related to the increased organic matter content rather than its value as a fertilizer. Composts provide a stabilized form of organic matter that improves the physical properties of soils by increasing nutrient and water holding capacity, total pore space, aggregate stability, erosion resistance, temperature insulation, and decreasing apparent soil density [12]. Application of MSS compost treated with lime has improved the chemical properties of soil by increasing soil pH (in acidic soils) and soil nutrient content [13].

On the other hand, accumulation of heavy metals by plants grown in MSS amended soil [13-15] can be a serious problem that requires a continuous monitoring. Risks of soil contamination when waste materials are used as fertilizer have been a matter of frequent concern [16]. There is a concern that heavy metals in the composted product may transfer from soil and accumulate in edible plants. Some of these heavy metals can be detrimental to human, plant or animal life if they are present above certain limits. Thuy et al. [17] indicated that heavy metals are one of the pollutants of most concern around the world. Elevated concentrations of heavy metals in harvested plant tissue could expose consumers to excessive levels of potentially hazardous chemicals [18]. Cadmium and Pb are the heavy metals of greatest concern to human health since plants can take them up and introduce them into the human food chain. Cadmium may accumulate in the human body and induce kidney dysfunction, skeletal damage and reproductive deficiency [19]. Elevated Cd concentrations in soil, resulting from the application of biosolids has been perceived as a potential environmental hazard [20, 21]. The primary Cd risk posed by the agricultural use of biosolids is the increased dietary Cd intake of people consuming crops grown on these soils. Lead and Ni are also possible contaminants in MSS. Lead causes liver, brain, and central nervous system dysfunction and is classified by the U.S. EPA as a probable human carcinogen [8]. According to the Institute of Medicine [22] and the Agency for Toxic Substances and Disease Registry [23], Ni can cause respiratory problems.

Although soil microorganisms require metals for growth and activity, heavy metals are toxic to soil microorganisms when present in excessive concentrations [24]. Increased concentrations of heavy metals in soil have shown negative impact on beneficial soil microorganisms as indicated by the activities of the enzymes they produce [7]. The rate of release of heavy metals from MSS into soil solution and subsequent uptake by plants could also result in phytotoxicity and/or bioaccumulation. Plant and animal cells have mechanisms for bioaccumulation (the selective and storage of great variety of molecules). This allows them to accumulate nutrients and essential minerals, but at the same time, they also absorb and store harmful substances such as heavy metals. Accordingly, toxins that are rather dilute in the environment can reach dangerous levels inside cells and tissues through this process of bioaccumulation. There is limited information on heavy-metal absorption by edible plants grown in biosolids-treated soil.

The objectives of this investigation were to: i) study the impact of mixing native agricultural soil with municipal sewage sludge (MSS) or yard waste (YW) incorporated with MSS (YW + MSS) on sweet potato (*Ipomoea batatas* L.) yield and quality; ii) determine the concentrations of seven heavy metals (Cd, Cr, Mo, Cu, Zn, Pb, and Ni) in sweet potato edible roots grown under three soil management practices (MSS, MSS+YW, and no-mulch soil); and 3) determine the concentration of seven heavy metal in sweet potato plant parts (leaves, stem, and feeder roots) under three soil management practices. Identifying soil management strategies that meet crop nutrition needs, support crop production, and protect food quality from excessive concentration of heavy metals was the focus of this investigation.

II. MATERIALS AND METHODS

A field study was conducted in summer 2008 on a Lowell silty-loam soil (2.6% organic matter, pH 7) located at Kentucky State University Research Farm, Franklin County, KY. The soil has an average of 12% clay, 75% silt, and 13% sand. Eighteen (18) standard plots of 22 × 3.7 m each were established. Plots were separated using stainless steel borders 20 cm above ground level to prevent cross contamination between adjacent treatments. The soil in six plots was mixed with municipal sewage sludge (MSS) obtained from Metropolitan Sewer District, Louisville, KY and used at 15 t $acre^{-1}$ (on dry weight basis). Six plots were mixed at 1:1 ratio with MSS and yard waste (MSS+YW) compost. Yard waste (YW) was obtained from Con Robinson Co., Lexington, KY and mixed with MSS at 15 t $acre^{-1}$ (on dry weight basis). The native soil in six plots was used as a no-mulch (NM) control treatment (roto-tilled bare soil) for comparison purposes. Plots were planted on June 13, 2008 with 9 weeks old sweet potato *Ipomoea batatas* cv. Beauregard seedlings obtained from Anderson County Farm Services (Lawrenceburg, KY) at 10 rows $plot^{-1}$ against the contour of the land slope, and irrigated by a uniform drip system.

A. *Sweet Potato Yield and Quality*

At harvest 3 plants were collected at random from each of the 18 field plots (six replicates for each soil treatment) and their roots were catagorized to U.S. No.1, U.S. petite No.1, U.S. commercial, and culls according the U.S. Standards for grades of sweet potatoes .

B. *Heavy Metal Analysis*

At harvest three plants were collected at random from each of the 18 field plots (six replicates for each soil treatment), washed with tap and deionized water and separated into edible roots, leaves, stem, feeder roots, and dried in an oven at 65^{O} C for 48 h [13]. The dried samples were ground manually with ceramic mortar and pestle to pass through a 1 mm non-metal sieve. Samples were re-dried to constant weight using an oven. To 1 g of each dry sample, 10 mL of concentrated nitric acid (HNO_3) trace metal grade was added and the mixture was allowed to stand overnight, and then heated for 4 h at 125^{O} C on a hot plate. The mixture was then diluted to 50 mL with double distilled water and filtered through filter paper No.1.

Native soil and soil incorporated with MSS and SS+YW mix were collected to a depth of 15 cm from field plots using a soil core sampler equipped with a plastic liner (Clements

Associates, Newton, IA, USA) of 2.5 cm i.d. Soil samples were oven-dried at 105^{O} C to a constant weight and sieved through a non-metal sieve to a size of 2 mm. Total metal concentrations in soil were determined as described above.

Since the total metal concentration in soils is not a very useful predictor of bioavailability of soluble concentrations of metal uptake by plants, the calcium chloride ($CaCl_2$) – extracted metal fraction was used to determine the readily soluble and extractable metals. Ten g dried soil samples were suspended in 25 mL of 0.01 $CaCl_2$ and heated at $90^{O}C$ on a hot plate for 30 min. The resulting supernatants were filtered hot through Whatman filter paper #42, and 2 drops of 1 M HNO_3 trace metal grade were added to prevent metal precipitation and to inhibit microbial growth in samples [25].

Concentrations of Cd, Cr, Ni, Pb, Zn, Cu, and Mo were determined using inductively coupled plasma-mass spectrometer (ICP-MS) following the U.S. EPA method 6020a [26] and using an octopole collision cell ICP-MS (7500cx, Agilent, Santa Clara, CA, USA). All standards were NIST traceable and were cross referenced to an extra NIST traceable source. All analytes were analyzed using ICP in standard mode except for Cr, for which the octopole was pressurized with He to eliminate $^{40}Ar^{12}Cr$ interferences for ^{52}Cr. Relative percent difference for replicate analyses was <3% and spike recovery ranged from 85-100%. Instrument detection limits (IDL) were 0.1016, 0.0755, 0.1259, 0.0883, 0.5324, 0.0113, and 0.0454 ng mL^{-1} for Cr, Ni, Cu, Zn, Mo, Cd, and Pb, respectively. The final m/z values used for quantification were 52, 60, 65, 66, 98, 112 and 208 for Cr, Ni, Cu, Zn, Mo, Cd and Pb, respectively. Elemental concentrations of soil and plants grown under three soil management practices were statistically analyzed using SAS procedure. Means were compared using Duncan's multiple range test [27].

III. RESULTS AND DISCUSSION

Total sweet potato marketable yield was greatest and weight of culls was lowest in MSS+YW treatment compared to MSS and NM treatments (Figure 1). This increase may be due to improved soil fertility, nutrient retention, soil porosity and water holding capacity. Increased crop yields are often attributed to increased organic matter content and improvements in the physical properties of the soil after the addition of composted materials. These include increased aggregate stability [28], increased moisture holding capacity, and reduced bulk density [29].

As previously described, plants can transfer and concentrate metals from soil. Regarding heavy metal bioavailability, Pb, Cd, and Ni are the heavy metals of greatest concern to human health since plants can accumulate them and introduce them into the food chain. Regardless of soil treatments, Pb concentrations were low in edible roots. Concentration of Pb in sweet potato stems of plants grown in YW+MSS was significantly higher than Pb concentrations in stems of plants grown in MSS alone or the NM soil (Figure 2, upper graph). These findings indicated that either the yard waste compost used in this investigation or the native soil that was incorporated with MSS, was the source of this high concentrations of Pb. Analyses of samples collected from premixed MSS, YW compost, and no much (NM) native soil revealed that Pb concentrations were 1.6, 3.8, and 2.2 µg g^{-1} dry weight, respectively, suggesting that YW compost could be the source of this Pb. Lead is defined by USEPA as potentially toxic to most forms of life. According to the Codex Alimentarius Commission of the Joint FAO/WHO Food Standards [30], the maximum level for Pb in most vegetables is 0.1 mg kg^{-1} on fresh weight basis. Lead is one of the many toxic metals in our environment [31]. Although Pb has no biological role in animals, plants, and microorganisms, it forms a bond with the sulfhydryl group of proteins, and hence can disrupt the metabolism and biological activities of many proteins and has caused cancer in kidneys of rodents.

Cadmium in edible sweet potato roots of plants grown in MSS-mixed soil was not detectable. Cadmium was significantly higher (0.14 µg g^{-1}) in edible roots grown in NM soil compared to YW+MSS mixed soil (Figure 2, middle graph). Human exposure to Cd has been associated with cancers of the prostate, lungs and testes [32]. Concentration of Ni was greatest in the edible roots of plants grown in YW+MSS treatments compared to other treatments (Figure 2, lower graph). There is a lack of US FDA guidance on Ni limits in food. Additional toxicological information on Ni and its level in edible crops are required. According to the State Environmental Protection Administration in China [33], the maximum permissible limits of Cd, Cr, Cu, Ni, Pb, and Zn for vegetables and fruits are 0.2, 0.5, 20, 10, 9, and 100 mg kg^{-1}, respectively on a dry weight basis.

Addition of MSS to agricultural soils is a management practice that can be exploited to replace synthetic fertilizers. However, potential bioaccumulation and mobility of heavy metals from MSS into growing plants could increase the potential transfer of heavy metals through crops to animals (feed crops) and humans (food crops) [34]. Heavy metals are not biodegradable and therefore can accumulate in human vital organs [35] and lead to progressive toxic effects if consumed with food that has concentrations of heavy metals above the permissible levels. Heavy metals are toxic to soil microorganisms when present in excessive concentrations [24]. In addition, plants themselves can also suffer from excess heavy metals in soil. Pandy and Sharma [36] indicated that the wilted appearance of Ni treated plants, when water was not limiting in the rooting medium, suggested impediment in facilitated movement of water from roots to the leaves. The same authors also indicated that the differences in the effectiveness of the heavy metals could possibly related to other biological reactivity in oxidation-reductions leading to generation of reactive oxygen species (ROS). The ROS are known to damage the integrity and functioning of cellular membranes thus affecting facilitated influx and the long-distance transport of water in plants.

Analyses of Cr and Cu in sweet potato plant parts revealed that the concentration of Cr was greatest in edible roots of plants grown in YW+MSS treatment compared to other treatments (Figure 3, upper graph). There were no significant differences among Cu concentrations (Figure 3, lower graph) and Zn concentration in sweet potato plant parts analyzed (Figure 4, upper graph). Although Zn has relatively low toxicity to humans, studies have shown allergies and Zn poisoning could occur along the food chain [37]. Molybdenum concentration was generally higher in plant tissues grown in

MSS treatments compared to NM treatments (Figure 4, lower graph). Molybdenum is the least abundant essential micronutrient found in most plant tissues and is required by selected enzymes (nitrate reductase, xanthine dehydrogenase, aldehyde oxidase and sulfite oxidase) to carryout redox reactions for enzyme activity [38].

The overall distribution of each of the seven heavy metals in each plant part, regardless of the soil treatment, is presented in Figure 5. While, no significant differences were found in Cd accumulation among plant parts analyzed, Pb was higher in the leaves and feeder roots and Ni was higher in edible and feeder roots. Except for Ni, Cr and Mo, concentrations in edible roots tended to be the lowest concentrations, compared to concentrations in other plant parts.

Reducing heavy metal pool for plant root uptake in soil can be achieved by naturally occurring or artificial additives such as lime material, clay, and organic matter [39]. Yuan and Lavkulich [40] reported that the adsorption capacity of a soil for Zn was reduced by 72%, when 11% of the organic carbon content was lost. Total Cu and Zn concentrations in sweet potato plant parts were high compared to other metals (Figure 5). Generally, Zn and Cu concentrations in MSS were lower than the permissible limits established by USEPA. Data for all heavy metals in sweet potato plant parts analyzed in this investigation are expressed on dry weight basis. Considering that water content of the sweet potato root was 86%, therefore, the Zn, Cu, Cd, and Pb concentrations were far below their Codex-established maximum limit of 0.1 mg kg^{-1} of sweet potato roots and they are of no major concern in the present study.

One might consider that the aggressive nitric acid extraction used in determining heavy metals greatly altered the soil chemical environment, dissolving much greater quantities of metals from soil solid phase than the amounts that are plant-available. Thus, nonaggressive solutions such as $CaCl_2$ extraction used in the present investigation, which extracted small quantities of heavy metals from soil, may measure the short-term bioavailable pool more directly than aggressive extractants. Accordingly, it is reasonable to use nonaggressive solutions that represent the natural behavior of metals in the soil solution. Extractable metals in soil using the mild $CaCl_2$ extraction procedure were generally low compared to the nitric acid extraction procedure (Figures 6-8). The strong and aggressive acidic nature of nitric acid caused large quantities of metals to be extracted from soils even when the plant availability of these metals was low because of either the near-neutral soil pH or high clay and organic matter content. This means that these extracted quantities are usually much in excess of quantities of metals taken up by crops (bioavailability) under natural field conditions on a time scale of growing seasons. Accordingly, acidic-extractable metals can be more directly correlated to total metals in soil than to plant-available metals.

In fact, the USEPA [41] has defined clean sludge in terms of its heavy metal content (mg kg^{-1}; Zn 1400, Cu 1500; Ni 420, Cd 39; Pb 300; Cr 1200; Mo 75). Unlimited amounts of sludge could be added to agricultural soil if these metals were below the USEPA limits. The concentrations of heavy metals in MSS used in this study were below the allowable limits and therefore this sludge has a great potential for agricultural use. However, soils in the agricultural or urban areas often serve as the major sink for heavy metals released into the environment from various anthropogenic sources. Accordingly, monitoring heavy metals in soil and edible plants should be regarded as a requirement for the safe use of soil amendments in agricultural fields.

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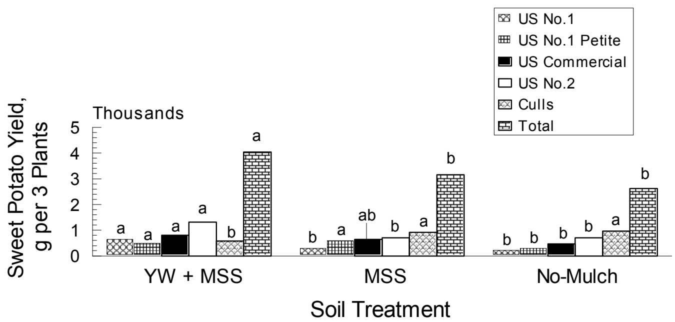

Fig. 1 Yield of sweet potato edible roots of plants grown under three soil management practices. Statistical comparisons were carried out between three soil management practices for each sweet potato class or total yield. US No.1 consists of sweet potatoes which are not more than 3.5” in diameter, firm, smooth, well-shaped, and free from disease or breakdown injury. US No. 1 petite are 1.5-2.25”in diameter with properties same as US No.1. US commercial are sweet potatoes which meet all the requirements of US No.1 grade except that an increased 2% defect is allowed. Sweet potato culls are soft, not smooth, not well-shaped, growth cracked, cuts, and sprouts. Bars accompanied by different letter(s) in each class or total yield are significantly different (P< 0.05) from each other using Duncan's multiple range test [27].

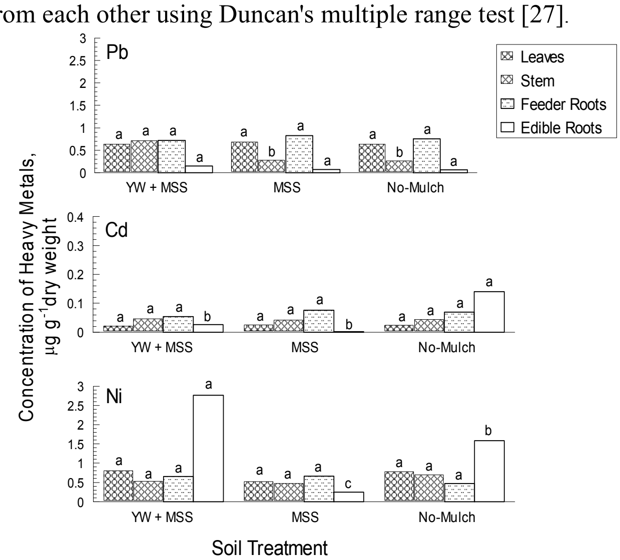

Fig. 2 Concentrations of Pb, Cd, and Ni in sweet potato plant parts. Plants were grown under three soil management practices. Statistical comparisons were carried out between three soil management practices for each plant part. Bars for each element accompanied by the same letter are not significantly different (P> 0.05) from each other using Duncan’s multiple range test [27].

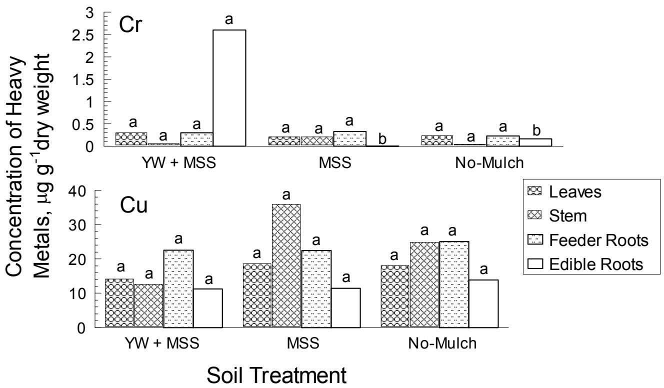

Fig. 3 Concentrations of Cr and Cu in sweet potato plant parts. Plants were grown under three soil management practices. Statistical comparisons were carried out between three soil treatments for each plant part. Bars for each element accompanied by the same letter are not significantly different ($P > 0.05$) from each other using Duncan's multiple range test [27].

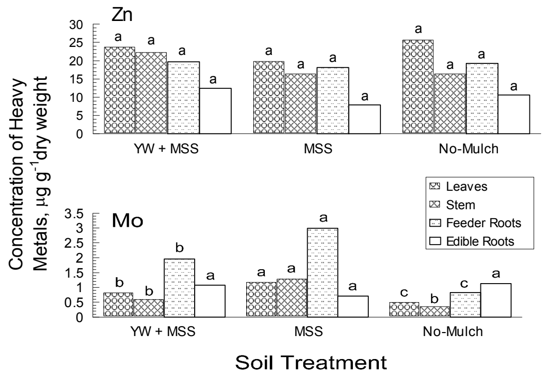

Fig. 4 Concentrations of Zn and Mo in sweet potato plant parts. Plants were grown under three soil management practices. Statistical comparisons were carried out between three soil treatments for each plant part. Bars for each plant part accompanied by the same letter(s) are not significantly different ($P > 0.05$) from each other using Duncan's multiple range test [27].

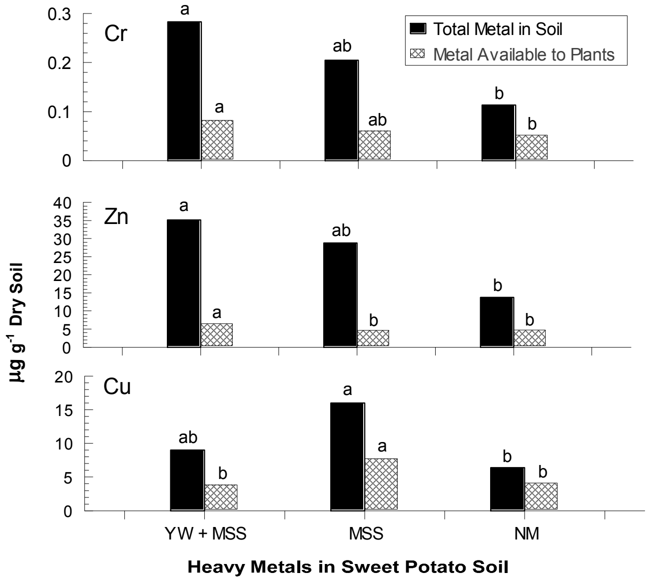

Fig. 5 Overall concentrations of seven heavy metals in sweet potato plant parts and total concentrations of each of the seven metals per g dry weight. Plants were grown under three soil management practices. Statistical comparisons were carried out between plant parts for each element. Bars for each element accompanied by the same letter are not significantly different ($P > 0.05$) from each other using Duncan's multiple range test [27].

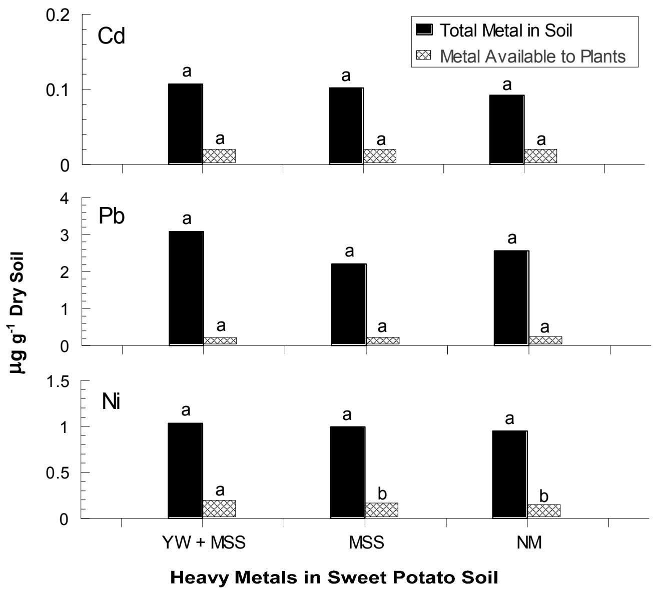

Fig. 6 Concentrations of total Cd, Pb, and Ni and quantities available to plants in soil collected from the rhizosphere of sweet potato plants grown under three soil management practices. Statistical comparisons were carried out between three soil treatments for each element. Bars for each total metal or metal available to plants accompanied by the same letter are not significantly different ($P > 0.05$) from each other using Duncan's multiple range test [27].

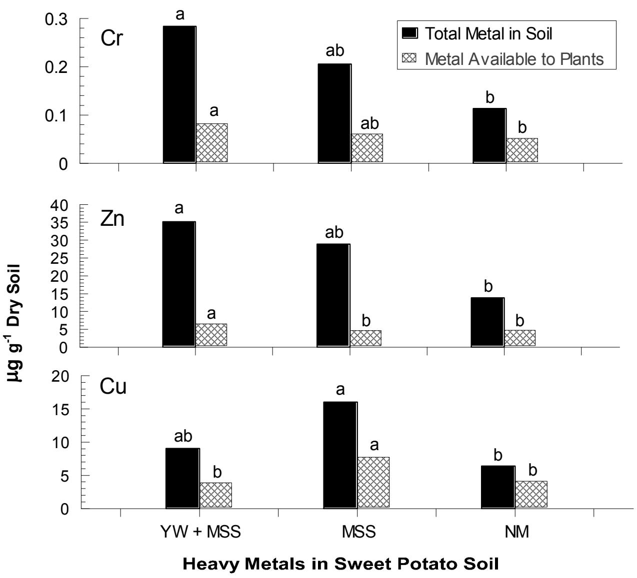

Fig. 7 Concentrations of total Cr, Zn, and Cu and quantities available to plants in soil collected from the rhizosphere of sweet potato plants grown under three soil management practices. Statistical comparisons were carried out between three soil treatments for each element. Bars for each total metal or metal available to plants accompanied by the same letter are not significantly different ($P > 0.05$) from each other using Duncan's multiple range test [27].

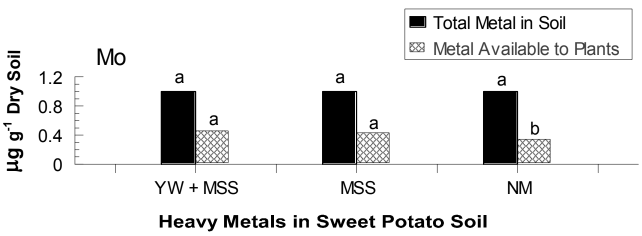

Fig. 8 Concentrations of total Mo and quantities available to plants in soil collected from the rhizosphere of sweet potato plants grown under three soil management practices. Statistical comparisons were carried out between three soil treatments for each element. Bars for each total metal or metal available to plants accompanied by the same letter are not significantly different ($P > 0.05$) from each other using Duncan's multiple range test [27].